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Spread of a Low-Fitness Drug-Resistant *Mycobacterium tuberculosis* Strain in a Setting of High Human Immunodeficiency Virus Prevalence▽

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The fitness cost associated with the evolution of resistance to rifampin in *Mycobacterium tuberculosis* may be different in clinical isolates compared to in vitro-generated mutants. An atypical Beijing strain (attenuated phenotype) demonstrated the ability to spread despite acquiring resistance to rifampin. Transmission was linked to human immunodeficiency virus coinfection ($P = 0.029$), raising concern for the spread of drug resistance in vulnerable populations.

The fitness of *Mycobacterium tuberculosis* strains circulating in a community is possibly the driving force perpetuating the tuberculosis epidemic. This is particularly true for the spread of drug resistance, since it has been suggested that the evolution of drug resistance has a fitness cost resulting in the overall attenuation of the pathogen (1). This phenomenon has been demonstrated during the spontaneous in vitro evolution of resistance to rifampin, where a direct correlation was observed between the frequency at which a specific nonsynonymous single nucleotide polymorphism (nsSNP) occurred and the fitness of the mutant clone (4). That study showed that rarely observed nsSNPs had a high fitness cost, whereas frequently observed nsSNPs had a low fitness cost. These results correlated well with the frequency of nsSNPs observed in rifampin-resistant clinical isolates (4).

Molecular epidemiological studies have suggested that certain *M. tuberculosis* genotypes are more successful than others (3, 5). Accordingly, it has been hypothesized that the Beijing genotype is a high fitness genotype, possibly as a result of the evolution of unique properties (2, 11, 16). Strains with the Beijing genotype can be broadly grouped as typical or atypical according to the presence or absence, respectively, of an IS*6110* insertion in the NTF region of the *M. tuberculosis* genome (9). Phylogenetic analysis has provided evidence that these two genotypes are derived from a common progenitor (6); however, they demonstrate vastly different epidemiological characteristics, since strains with the atypical Beijing genotype are only rarely observed (9, 14). This has prompted speculation that atypical Beijing strains have lower fitness than typical Beijing strains. We hypothesize that rifampin resistance causing nsSNPs that have a fitness cost would only be rarely observed in *M. tuberculosis* strains with an atypical Beijing genotype unless epidemiological factors favoring their spread were present.

To test this hypothesis, sputum specimens were collected from retreatment cases attending health care clinics or referral hospitals in two provinces in South Africa. Each specimen was subjected to routine culture-based drug susceptibility testing for isoniazid and rifampin. During the period from January 2001 to October 2004, 312 rifampin-resistant cases were identified from the Western Cape (WC) region (12), while during the period from September 2003 to May 2004, 117 rifampin-resistant cases were identified from the Eastern Cape (EC) region. DNA sequence analysis of the rifampin resistance-determining region (RRDR) of the *rpoB* gene (13) showed that >90% of rifampin-resistant isolates had an nsSNP in the RRDR region. Of the 30 nsSNPs identified, 25 nsSNPs appeared at a frequency consistent with frequencies reported in in vitro-generated rifampin-resistant mutants (4, 7, 10) (Table 1). The frequency of appearance of the remaining five nsSNPs was discordant compared to the in vitro-generated rifampin-resistant mutants (Table 1). The nsSNPs at codons 516 (GAC→GTC and GAC→TAC) and 533 (CTG→CCG) were significantly under-represented in the in vitro-generated rifampin-resistant mutants, while nsSNPs at codon 522 (TCG→TTG) and codon 526 (CAC→CGC) were significantly over-represented in the in vitro-generated rifampin-resistant mutants (Table 1). This suggests that the nsSNPs at codons 516 (GAC→GTC and GAC→TAC) and 533 (CTG→CCG) had a lower fitness cost in clinical isolates compared to in vitro-generated rifampin-resistant mutants. Conversely, the nsSNPs at codons 522 (TCG→TTG) and 526 (CAC→CGC) appear to have a high fitness cost in clinical isolates.

In order to determine whether a relationship existed between strain genotype and the nsSNPs conferring rifampin

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TABLE 1. Distribution of nsSNPs conferring resistance to rifampin in clinical isolates from the WC and EC regions of South Africa[a]

Codon	Sequence (wild type→ mutation)	No. of isolates								
		In vitro Rif[r] studies combined[b] (n = 368)	WC Rif[r]				EC Rif[r]			
			All (n = 312)	NB (n = 196)	Beijing (n = 116)		All (n = 117)	NB (n = 58)	B (n = 59)	
					T (n = 101)	A (n = 15)			T (n = 14)	A (n = 45)
490	CAG→CAA									
511	CTG→CCG				2	1			3	
512	CAA→AAA		3	3						
513	CAA→AAA	2	4	4						
	CAA→CCA		1			1				
	CAA→GAA	4								
	CAA→CTA	2								
516	GAC→GTC	3[c]	15	7	5	3	29	1	1	27
	GAC→TAC		15	13	1	1	6	2	1	3
	GAC→TTC		1	1						
	GAC→GGC		1		1					
519	AAC→AAG	1								
522	TCG→TTG	46[c]								
526	CAC→AAC		3	1	1	1	1	1		
	CAC→CTC	1	5	4	1		1		1	
	CAC→TAC	58	19	14	5		6	3	1	2
	CAC→GAC	18	8	7	1					
	CAC→GCC		2	2						
	CAC→TGC		1	1						
	CAC→CGC	57[d]	1	1						
	CAC→CCC	4								
529	CGA→CCA	1								
	CGA→CAA	1								
	CGA→GGA	1								
531	TCG→TGG	17	8	2	3	3	2	2		
	TCG→TTG	132	169	110	54	5	58	42	5	11
	TCG→CAG		3		3					
	TCG→TTC		1		1					
533	CTG→CCG	1[c]	15	5	10					
	CTG→GAG		2		2					
Multiple nsSNPs		2	1	1						
Insertions		1	1	1						
Deletions		15[e]	2	2						
nsSNPs absent from RRDR		1	27	16	11		11	7	2	2

[a] Rif[r], rifampin resistant. NB, non-Beijing; B, Beijing; T, typical; A, atypical.
[b] Data were combined from previously published sources (4, 7, 10).
[c] Under-represented in the in vitro generated rifampin-resistant mutants (z-test [$P < 0.001$]).
[d] Over-represented in the in vitro-generated rifampin-resistant mutants (z-test [$P < 0.001$]).
[e] Eleven different deletion events (7, 10).

resistance, the isolates from this study were classified as either Beijing or non-Beijing by spoligotyping (8). The results showed that 116 (37%) of the rifampin-resistant cases from the WC region and 59 (50%) of the rifampin-resistant cases from the EC region were infected with a Beijing genotype strain (Table 1). Subclassification of the Beijing isolates as either typical or atypical (6) showed that the population structure of rifampin-resistant Beijing strains was significantly different in the two study settings (Fisher exact test odds ratio = 21.6; 95% confidence interval = 9.6 to 48.6, $P < 0.0001$) (Table 1). The nsSNP at codon 516 (GAC→GTC) was associated with the atypical Beijing genotype from the EC (Fisher exact test odds ratio = 45; 95% confidence interval = 3.8 to 525, $P = 0.0008$), while the nsSNP at codon 533 was mostly found in isolates with the typical Beijing genotype from the WC (Table 1).

IS*6110* DNA fingerprinting (15) showed that isolates from the WC region with the typical Beijing genotype and either an nsSNP at codon 516 (GAC→GTC) or codon 533 (CTG→CCG) were not clustered (5 of 5 banding patterns and 9 of 10 banding patterns, respectively), thereby suggesting that these nsSNPs had evolved independently and that the resulting clones were not transmitted. In contrast, the isolates from the EC region with the atypical Beijing genotype and a nsSNP at codon 516 (GAC→GTC) were clustered and also shared the rare −17 *inhA* promoter mutation (G→T) (data not shown), suggesting ongoing transmission. These isolates clustered with the atypical Beijing strains from the WC which had a nsSNP at codon 516 (GAC→GTC) and the −17 *inhA* promoter mutation, suggesting interprovincial spread. This finding was contrary to previous reports, which suggested that atypical Beijing strains are attenuated in their ability to transmit (9, 14), while the mutation at codon 516 (GAC→GTC) would have been expected to further compromise the ability of these strains to transmit unless compensatory mutations were present or the

epidemiological context allowed transmission to occur. Analysis of the host population in the EC region showed human immunodeficiency virus (HIV) coinfection to be a risk factor for the spread of the atypical Beijing strains (z-test for the hypothesis that a proportion of HIV-positive cases = 0.42, P = 0.029). In contrast, the frequency of atypical Beijing strains was low in the WC region, which in turn has a low incidence of HIV-tuberculosis coinfection (6). This raises concern for the spread of all drug-resistant strains in vulnerable populations.

In summary, greater vigilance is required to contain the drug-resistant tuberculosis epidemic in high-HIV-prevalence settings. This can be achieved by the development and implementation of rapid diagnostics, the provision of appropriate therapy, ensuring treatment adherence, and intensified screening of contacts. However, in order for diagnosis and treatment to be effective, it is essential that communities are educated to improve health-seeking behavior.

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